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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> A61K 31/765, 7/42, 7/48, C08F 16/34, 16/38, C08L 29/00, 29/14, A01N 35/02, A61L 2/18		<b>A1</b>	<b>(11) International Publication Number:</b> WO 00/03723 <b>(43) International Publication Date:</b> 27 January 2000 (27.01.00)
<b>(21) International Application Number:</b> PCT/AU99/00578 <b>(22) International Filing Date:</b> 16 July 1999 (16.07.99)  <b>(30) Priority Data:</b> PP 4719 17 July 1998 (17.07.98) AU PP 5167 10 August 1998 (10.08.98) AU  <b>(71) Applicant (for all designated States except US):</b> CHEMEQ LIMITED [AU/AU]; Petroleum House, Suite 8, 3 Brodie Hall Drive, Technology Park, Bentley, W.A. 6102 (AU).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> MELROSE, Graham, John, Hamilton [AU/AU]; 20 Nardina Crescent, Dalkcith, W.A. 6009 (AU). HUXHAM, Andrew, James [AU/AU]; 30 Stedham Way, Balga, W.A. 6061 (AU).  <b>(74) Agent:</b> WRAY & ASSOCIATES; 239 Adelaide Terrace, Perth, W.A. 6000 (AU).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> POLYMERIC COMPOUNDS AND METHODS OF FORMULATING SAME			
<b>(57) Abstract</b>  A method for the preparation of compositions of poly(2-propenal, 2-propenoic acid), whereby the compositions exhibit one or more of the properties of increased stability, increased antimicrobial activity, reduced trans-dermal migration of low molecular weight components of the composition, and the formation of continuous antimicrobial film on substrates, the property or properties making the composition suitable for one or more of antimicrobial use, dermatological use, and/or use as an animal feed additive.			

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TITLE

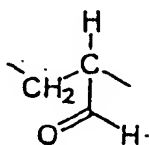
"POLYMERIC COMPOUNDS AND METHODS OF FORMULATING SAME"

FIELD OF THE INVENTION

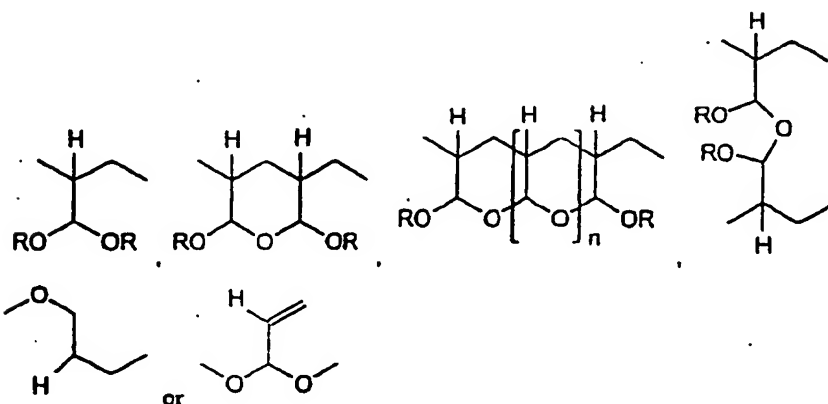
The present invention relates to polymeric compounds and methods of  
 5 formulating same, said polymeric compounds having a polyacrolein sub-unit in  
 aldehyde, hydrated, hemi-acetal or acetal form and having biostatic or biocidal  
 properties. More particularly, the present invention is directed to compositions  
 containing the above noted polymeric compounds and the biostatic and/or  
 biocidal uses of these compositions.

10 BACKGROUND ART

The broad-based antimicrobial properties of polymers having the repeating  
 polymeric unit:



15 ; or this unit in its hydrated, hemi-acetal or acetal form, represented by the  
 formulae:



wherein R is hydrogen or alkyl and n is an integer of one or more have been demonstrated previously (International Patent Application Publication WO 88/04671). The compounds particularly described therein include poly(2-propenal, 2-propenoic acid).

5 It has also been noted previously (International Patent Application Publication WO 96/38186) that poly(2-propenal, 2-propenoic acid) is formed when the aldehyde groups of poly (2-propenal) *syn* polyacrolein are partially auto-oxidised to carboxyl groups. It was further noted that the polymer is soluble in dilute aqueous bases, for example aqueous sodium carbonate.

10 It is known that antimicrobial compositions may be used as preservatives, or as the active ingredients in disinfectants, dermatological compositions including sun screen formulations or antiseptic formulations, or in animal feed additives. Generally these antimicrobial compositions must:

- be stable;
- 15 • be efficacious in killing micro-organisms within a specified time;
- be safe, that is be reasonably free of toxicity which may be caused by the trans-dermal migration of low molecular weight ingredients into the blood-stream so as to manifest toxicity, antigenicity, allergy, irritation or inflammation;
- 20 • have minimal odour; and
- in some dermatological preparations, have the property of sun screening and minimise adverse dermatological effects from the generation of free-radicals.

Specifically, antimicrobial formulations applied to inanimate objects and the skin are usually termed disinfectants and antiseptics, respectively. Often, regulatory  
25 standards demand that a disinfectant formulation first, is stable and secondly,

kills a chosen quantum of vegetative micro-organisms within 10 minutes. That is, the antimicrobial activity of these compositions must be biocidal and quick. The formulations described herein are substantially aimed at these goals, but often achieve more, for example killing even extremely resistant bacterial spores within  
5 the frequent standard of 24 hours.

Formulation of poly(2-propenal, 2-propenoic acid) simply by dissolution in dilute aqueous sodium carbonate, and then neutralisation to pH 7, has now been found to provide a composition that does not always kill micro-organisms fast enough to meet the above standards.

10 It is one object of the present invention to provide methods of preparing compositions containing compounds of the type described by the prior art and in particular poly(2-propenal, 2-propenoic acid), and which are useful disinfectants and/or antiseptics meeting these standards.

It is a further object of the present invention to provide a method of preparing  
15 polymers and/or copolymers derived from acrolein in accordance with the above mentioned prior art for use as useful disinfectants and/or antiseptics.

It is a still further object of the present invention to provide a method of preparing polymers and/or copolymers derived from acrolein in accordance with the above mentioned prior art, for use in dermatological formulations, including sunscreens.

20 It is yet still a further object of the present invention to provide a method of preparing polymers and/or copolymers derived from acrolein in accordance with the above mentioned prior art for use in other applications including as a preservative or as an animal feed-additive.

Throughout this specification, unless the context requires otherwise, the word  
25 "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

## DISCLOSURE OF THE INVENTION

In accordance with the present invention there is provided a method for the preparation of compositions of poly(2-propenal, 2-propenoic acid), whereby the compositions exhibit one or more of the properties of increased stability, increased antimicrobial activity, reduced trans-dermal migration of low molecular weight components of the composition, and the formation of continuous antimicrobial film on substrates, the property or properties making the composition suitable for one or more of antimicrobial use, dermatological use, and/or use as an animal feed additive.

- 5 In one form of the invention the composition exhibits increased stability, the method comprising the acidification of compositions of poly(2-propenal, 2-propenoic acid) to which has been added one or more anionic surfactant and/or one or more phenol.

- 15 The composition containing poly(2-propenal, 2-propenoic acid) may be firstly stored in basic composition.

Preferably, the anionic surfactant is selected from either sodium lauryl sulphate or disodium decyl (sulfophoxy) benzene sulfonate and disodium oxybis (decylsulfophenoxy) benzene sulfonate. Preferably, the phenol is o-phenylphenol.

- 20 In another form of the invention the composition exhibits increased antimicrobial activity, the method comprising preparation of the poly(2-propenal, 2-propenoic acid) in the presence of air and/or oxygen, with or without inhibitor.

- 25 Preferably, the method comprises the making of the composition of poly(2-propenal, 2-propenoic acid) into a basic composition. The composition may further comprise one or more of the group: ethylene diamine tetra acetic acid, a lower alkanol, a phenol, isothiazolinones and glutaraldehyde. The odour of any phenols and/or glutaraldehyde present in the composition is reduced by the presence of the poly(2-propenal, 2-propenoic acid).

In a further form of the invention the composition exhibits reduced trans-dermal migration of low molecular weight components of the composition as a result of the presence of poly(2-propenal, 2-propenoic acid). The low molecular weight composition may contain a sunscreen agent, for example either or both of octyl methoxy cinnamate and octyl dimethyl p-aminobenzoate.

In a still further form of the invention the composition, especially for dermatological use, exhibits a suncreening effect as a result of the presence of poly(2-propenal, 2-propenoic acid).

In yet a further form of the invention the composition exhibits the formation of a continuous antimicrobial film on substrates, the method comprising the combination of poly(2-propenal, 2-propenoic acid) together with anionic surfactant and/or a phenol.

Preferably, the surfactant is sodium lauryl sulphate or disodium decyl (sulfophoxy) benzene sulfonate and disodium oxybis (decylsulfophenoxy) benzene sulfonate. The phenol is preferably o-phenyl-phenol.

In a still yet further form of the present invention the composition, especially for dermatological use, exhibits a free-radical screening effect as the result of the presence of poly(2-propenal, 2-propenoic acid).

In accordance with the present invention there is further provided a composition adapted for antimicrobial uses and/or dermatological uses and/or uses as an animal feed additive, the composition comprising poly(2-propenal, 2-propenoic acid) and one or more of the following: an anionic surfactant, a phenol, ethylene diamine tetra acetic acid, a lower alkanol, isothiazolinones, glutaraldehyde, sunscreen agent, and an absence of low molecular weight components of the composition.

In one form of the invention the composition comprises an anionic surfactant chosen from either sodium lauryl sulphate or disodium decyl (sulfophoxy) benzene sulfonate and disodium oxybis (decylsulfophenoxy) benzene sulfonate. The phenol is preferably o-phenyl-phenol.

**BEST MODE(S) FOR CARRYING OUT THE INVENTION**

It has now been discovered that modifying the preparation of poly(2-propenal, 2-propenoic acid) as described in both WO 88/04671 and WO 96/38186 (Example 1b in each) by concurrently bubbling in air and/or oxygen during the polymerisation, produces a polymer with more discreet crystalline form which aids recovery and subsequent drying, and in solid or in liquid medium has less contaminating and slightly odorous oligomers, and has higher inherent rate of antimicrobial activity; see Example 1 hereinafter.

It has now been found that whilst basic aqueous compositions containing the poly(2-propenal, 2-propenoic acid) are biostatic and/or biocidal; nevertheless, the compositions are appreciably unstable. Although, it has further been shown that lowering the pH (lowering the hydroxyl ion concentration) of such compositions/solutions increases their chemical stability, counter-productively, it has been found that acidification of the composition to pH's below approximately pH 6, causes precipitation of the poly(2-propenal, 2-propenoic acid).

It has now been shown that this precipitation can be avoided until approximately pH to 3.5 (ie. over ten-fold, less hydroxyl ion concentration), by a method of formulating in which the poly(2-propenal, 2-propenoic acid) is first dissolved in dilute aqueous base, then anionic surfactant added, before the acidification. Useful anionic surfactants are either sodium lauryl sulphate ("SLS") or disodium decyl (sulfophoxy) benzene sulfonate and disodium oxybis (decylsulfophenoxy) benzene sulfonate in equal weight ratios with the poly(2-propenal, 2 propenoic acid). To be effective, it is important to maintain this order of addition, so that the poly(2-propenal, 2-propenoic acid) is in its anionic form before the addition of the surfactant; see Example 2 hereinafter. This apparent interrelationship between the two negatively charged species from poly(2-propenal, 2-propenoic acid), and anionic detergent is surprising since repulsion between the like-charges on the species would be expected.



It has now been found that basic aqueous solutions of poly(2-propenal, 2-propenoic acid) kill micro-organisms more rapidly than do acidic solutions of the polymer. This discovery led to the further finding that formulations of more stable, acidic compositions containing poly(2-propenal, 2-propenoic acid) and preferably, anionic surfactant, could subsequently be made basic and hence, more antimicrobially active, immediately before use as, for example, either a disinfectant and/or antiseptic and/or preservative; see Example 10 hereinafter.

It has now been shown that if a solution of poly(2-propenal, 2-propenoic acid) which contains a phenol, with anionic surfactant is acidified, a surprisingly stable emulsion is formed, and in this heterogenous system, the poly(2-propenal, 2-propenoic acid) in the hydrophobic phase is protected from chemical degradation by the hydroxyl ions in the hydrophilic phase; see Example 2 hereinafter. o-Phenyl-phenol is particularly useful, and in equal weight ratio with the poly(2-propenal, 2-propenoic acid).

It has now been shown that the inclusion of phenols additionally to anionic surfactants in compositions containing poly(2-propenal, 2-propenoic acid) allows further acidification of these compositions before any precipitation of the poly(2-propenal, 2-propenoic acid) ie. further chemical stability with respect to hydroxyl ion and/or base is achieved; see Example 2 hereinafter. Further, it has now been shown that if the composition containing poly(2-propenal, 2-propenoic acid) only is firstly stood, for example for about 11 days at about pH 9, in an aqueous alkaline medium, before the addition of the anionic surfactant and then phenol - precipitation will not occur at all, upon acidification; see Example 2 hereinafter.

It has now been shown that co-formulation of the composition containing poly(2-propenal, 2-propenoic acid), with anionic surfactant, and optionally with additionally a phenol, produces a composition which provides a continuous antimicrobial film upon substrates, after application thereto, eg. skin, flooring, walls, furniture etc. Without these additives, the film is invariably discontinuous and hence only partially protects the substrate, antimicrobially. It has now been shown that these films retain moisture, facilitating their protective antimicrobial

activities. If they also contain a volatile component which effects their pH-dependent antimicrobial activities, then, in turn, these activities may be increased as evaporation takes place; see Example 10 hereinafter.

5 It has now been shown that inclusion of anionic surfactant in formulations containing poly(2-propenal, 2-propenoic acid), increases their chemical stability to hydroxyl ion and/or base; see Example 3 hereinafter.

10 It has now been shown that poly(2-propenal, 2-propenoic acid) absorbs in the UV, and that a peak about 268 nm, as it is replaced by absorption at approximately 232 nm, correlates with and is a convenient monitor of the chemical stability of poly(2-propenal, 2-propenoic acid) in basic aqueous solutions; see "Stability Test" hereinafter.

15 It has now been shown that co-formulating anionic surfactant and/or ethylenediaminetetraacetic ("EDTA") acid and/or its salts and/or a lower alkanol enhances the antimicrobial properties of compositions containing the poly(2-propenal, 2-propenoic acid); see Examples 4 and 5 hereinafter.

20 It has now been shown, further, that not only is the antimicrobial activity increased - but surprisingly, it is *synergistically* increased by incorporating in the compositions containing the poly(2-propenal, 2-propenoic acid) either, EDTA (and/or its salts), and/or phenols, and/or isothiazolinones, and/or glutaraldehyde; see Example 7 hereinafter.

It has now been shown that inclusion of poly(2-propenal, 2-propenoic acid) decreases the notorious odours of compositions containing antimicrobial phenol(s) and/or glutaraldehyde; see Example 6 hereinafter.

25 It has now been shown that the surprising apparent interaction between the negatively-charged phenols and the negatively-charged anionic form of poly(2-propenal, 2-propenoic acid) is more general, and is thought to result from the interaction of the hydrophobic portions of the respective species. Thus, the

presence of poly(2-propenal, 2-propenoic acid) in an emulsion with the UV sunscreen octyl methoxy cinnamate, or octyl dimethyl p-aminobenzoate, is shown to prevent the migration of either of the sunscreens through a membrane which is a model for the skin; see Example 8 hereinafter. Hence, it is apparent that poly(2-propenal, 2-propenoic acid) may be used as a co-formulant to reduce potential toxicity and/or allergy and/or antigenicity and/or irritation and/or inflammation from sunscreen agents, or other compounds, eg. phenols, which results from trans-dermal migration of such constituents, in dermatological preparations, into the blood-stream.

10 It has been shown further, that the peak at 268 nm and below provides significant UVC absorption. UVC energy is greater than 50% of the energy in sunlight (Lide, D.R., "CRC Handbook of Chemistry and Physics", CRC Press, 73rd edition, 1992-93, page 14-8) and is in the wavelength to which the skin is most sensitive ("Harry's Cosmeticology", J.B. Wilkinson and R.J. Moore Eds., Chemical Publishing Co. Inc., New York, 1982, page 228) and which induces changes that  
15 alter the structure of DNA (Kano R.J. and Colome J.S. "Microbiology", West Publishing Company, 1986, page 162). Thus, it has now been found that it is an advantage to include poly(2-propenal, 2-propenoic acid) in dermatological sunscreen preparations.

20 It has now been shown that compositions containing poly(2-propenal, 2-propenoic acid) and containing only polymeric ingredients (eg. polymeric solvents/emollients and/or polymeric surfactants and/or emulsifiers) and/or volatile ingredients provide compositions for dermatological applications, free of any components which, after spreading on the skin, may migrate trans-dermally  
25 and into the bloodstream to cause toxicity, allergy etc; see Example 8 hereinafter.

It has now been shown that the formulation of such compositions (eg free of conventional surfactants) is facilitated by the surfactant properties of poly(2-propenal, 2-propenoic acid).

It has now been shown that poly(2-propenal, 2-propenoic acid) has the capacity to absorb free-radicals and this is a distinct use in dermatological compositions with a view to minimising skin-damage brought about by free-radicals; see Example 9 hereinafter.

- 5 It has now been shown that the methods, compositions and uses provided herein, apply to all compounds described in WO 88/04671 and WO 96/38186, particularly those compounds which are hydrophilic and/or soluble in aqueous media; the methods shown herein of keeping these compounds in solution and/or emulsion in aqueous media generally facilitate any desired chemical reactions
- 10 with these compounds in acidic media.

The invention will now be described with reference to a number of specific Examples, each of which should not be construed as limiting the scope of the invention. In the following examples, reference is made to a number of tests as follows:

15 1. Biocidal Test

Dilute sample with sterile water to obtain required concentration. Weight 19.9g of diluted sample into a sterile jar and inoculate with 0.1 mL of  $10^7$  -  $10^8$  suspension of *Ps. aeruginosa* and mix. Immediately transfer 1 mL of inoculated sample to 9 mL of letheen broth and vortex. Plate out serial 1 in 10 dilutions. Pour with

20 tryptone soya agar. Incubate 3 days at 37°C.

2. Minimal Kill Concentration Test

Make serial 1 in 2 dilutions of the sample using sterile 0.85% saline. Add 0.1 mL of suspension of test organism to the dilution. Incubate at 37°C for 24 hours. Subculture 1 mL from each tube into 10 mL sterile nutrient broth plus TWEEN 80;

25 incubate at 37°C for 24 hours.

### 3. Sporicidal Test

Add 1 mL of spore suspension of *B. subtilis var niger* (cfu  $10^7$ /mL) to 10 mL of solution of the sample in a sterile bottle, and vortex. Immediately remove 0.020 mL and add to recovery broth; repeat 5 times. Vortex and incubate at 37°C for 14 days. Confirm by "heat shock" to all tests.

### 4 Sporicidal Efficacy Test

Inoculate sterile glass slides with a suspension of *B. subtilis var niger* (cfu  $10^7$ /mL). Dry under vacuum for 24 hours at 30°C. Add 4mL sample solution to slide. After 10 min contact time, dry slides at 30°C for 72 hours. Sonicate and vortex slides into deactivation broth. Enumerate by performing 4 serial 1 in 10 dilutions in agar and incubate for 48 hours at 30°C.

### 5. Modified Kelsey Sykes Test

Add 1 mL of culture of test organism ( $2 \times 10^8$  -  $2 \times 10^9$ ) cfu/mL containing 1% yeast to 3 mL of test solution. At 8 minutes, subculture 0.02 mL into each of 5 tubes containing recovery broth, and vortex. Incubate at 37° C for 48 hours.

### 6. Stability Test

Poly(2-propenal, 2-propenoic acid) (1 g) was dissolved in 0.5% w/w aqueous sodium carbonate, and stood at room temperature. The stability was measured by the UV method.

The stability of aqueous solutions of polymers were followed by the disappearance of a UV peak near 268nm, and the appearance of a peak near 232nm; ca. absorbance at 268nm, of 0.02% solution = 1.5.

Example 1(a) With hydroquinone; open to air

Water (720 mL at ambient temperature, about 20°C) and acrolein (60 g; freshly distilled and hydroquinone added to 0.25%w/w) were placed in an open beaker, within a fume cupboard, and very vigorously stirred, mechanically. Then, 0.2 M aqueous sodium hydroxide (21.4 mL) was added to bring the pH to 10.5 - 11.0. The solution immediately turned a yellow typical of the hydroquinone anion and, within a minute, the colour had disappeared and the clear solution became milky. About 1 minute later, precipitation of a white crystalline, flocculent polymer began, and appeared complete within 15 - 30 minutes. The polymer precipitate, poly(2-propenal, 2-propenoic acid), was filtered and washed with water (250 mL), dried at room temperature upon filter papers for 2 days (yield 25.2 g), then spread as a thin layer in glass petri dishes and heated at 40°C/8 hours. This heating was continued at the following schedules: 50°C/15 hours (then ground), 65°C/4 hours, 70°C/2 hours, 75°C/18 hours, 82°C/24 hours. It is envisaged that this method may be scaled-up to include, eg. the stepwise addition of acrolein, followed by more rapid drying.

Typically a solution of the resulting poly(2-propenal, 2-propenoic acid) was prepared by adding 2 g, with stirring over 15-30 minutes, to a 1% w/w aqueous sodium carbonate solution (100 mL) and diluted as required.

(b) With hydroquinone; closed vessel

As above for (a) except the reaction vessel was a stoppered 1 litre flask; this resulted in a slowly-forming precipitate which was glassy and poorly crystalline - yield 25.5 g.

(c) Without hydroquinone; closed vessel

As above for (a), except hydroquinone was excluded, and the reaction vessel was a stoppered 1 litre flask; this resulted in a crystalline product - yield 21.0 g.

(d) Without hydroquinone; open to the air

- 5 As above for (a), except hydroquinone was excluded; this resulted in a crystalline product - yield 26.0 g.

After 3 days at room temperature, the diluted samples (0.25% w/w), being the product of examples 1(a) to 1(b), were tested by the Biocidal Test. The results are shown in Table 1:

10

Table 1

cfu/mL (i.e. Colony forming units/mL)

<u>Sample</u>	<u>1 minute</u>	<u>5 minutes</u>	<u>15 minutes</u>	<u>30 minutes</u>
1 (a)	$6.6 \times 10^5$	$1.6 \times 10^5$	50	0
1 (b)	$7.7 \times 10^5$	$4.7 \times 10^5$	$8.2 \times 10^3$	0
1 (c)	$9.0 \times 10^5$	$8.1 \times 10^5$	$6.2 \times 10^3$	0
1 (d)	$8.3 \times 10^5$	$2.1 \times 10^5$	60	0

Example 2

- 15 A series of tests were conducted to examine the impact of surfactant, and/or water, and/or buffer, and/or phenol on precipitation state and pH. Results are shown in Table 2:

Table 2

<u>Polymer</u>	<u>Surfactant</u>	<u>Water</u>	<u>Buffer</u>	<u>Phenol</u>	<u>Observation at 3 days</u>	<u>pH at precipitation</u>
10mL A		2mL	4mL I	-	heavy precipitate	5.5
10mL A	0.4g C	-	4mL I	-	clear	3.5
10mL A	0.4g D	-	4mL I	-	clear	3.5
10mL B	0.4g D	-	4mL I	-	clear	<3.5
10mL A	0.4g E	-	-	-	clear	<3.5
10mL A	0.4g E	-	-	0.2g F	emulsion	<3.5
10mL A	0.4g E	-	-	0.4g G	emulsion	<3.5
10mL A	0.4g E	-	-	0.4g H	emulsion	<3.5

- A - 4% w/w polymer in 4% w/w sodium bicarbonate, freshly prepared.  
 B - as A, aged for 11 days at room temperature.  
 5 C - Aqueous 4% w/w sodium lauryl sulphate.  
 D - Aqueous 4% w/w Dowfax 3B2.  
   - Decyl (sulfophenoxy) benzenesulfonic acid, disodium salt.  
   - Oxybis (decylbenzenesulfonic acid), disodium salt.  
 10 E - Dowfax 3B2 - Aqueous 4% w/w Dowfax 3B2.  
   - Decyl (sulfophenoxy) benzenesulfonic acid, disodium salt.  
   - Oxybis (decylbenzenesulfonic acid), disodium salt.  
 F - Dowicide A - orthophenylphenol, sodium salt.  
 G - Dowicide (orthophenylphenol), 33% w/w, in sodium hydroxide solution, 66% w/w.  
 15 H - 4-tert-amylphenol, 33% w/w, in sodium hydroxide solution, 66% w/w.  
 I - 10% w/w acetic acid : sodium hydroxide buffer, pH 4.5.

Example 3

- 20 (a) A solution of 2% w/w poly(2-propenal, 2-propenoic acid) in 2% w/w aqueous sodium carbonate containing 2% w/w sodium lauryl sulphate at ambient pH (~9.8) was shown by the UV test to be more stable than a solution without the sodium lauryl sulphate over 11 days/38°C.
- 25 (b) A solution of 4% w/w poly(2-propenal, 2-propenoic acid) in 4% w/w aqueous sodium bicarbonate containing 2% w/w sodium lauryl sulphate, acidified



with hydrochloric acid to pH5, was shown by the UV test to be more stable than a solution without the sodium lauryl sulphate over 4 days/room temperature.

5 Results are shown in Table 3:

Table 3

Sample	Sodium Lauryl Sulphate (2% w/w)	pH	Absorbance Ratio $\frac{A_{232}}{A_{268}}$		
			0hrs	96hrs	264hrs
3(a)	No	9.8	0	-	$\infty$
3(a)	Yes	9.8	0	-	1.36
3(b)	No	5.0	0	1.05	-
3(b)	Yes	5.0	0	0	-

Example 4

10

A series of tests were conducted to examine the impact of the incorporation of EDTA or SLS on the antimicrobial activity of a 2% w/w solution of poly(2-propenal, 2-propenoic acid). Results of the Biocidal Test are shown in Table 4:

15.

Table 4

cfu/mL

<u>Sample</u>	<u>1 minute</u>	<u>5 minutes</u>	<u>15 minutes</u>	<u>30 minutes</u>
Control	$3.7 \times 10^6$	$5.1 \times 10^6$	$9.2 \times 10^4$	0
plus EDTA (0.25%)	$3.7 \times 10^6$	0	0	0
plus SLS (0.25%)	$3.7 \times 10^6$	$2.7 \times 10^5$	0	0

Example 5

A test formulation of 1.5% w/w solution of poly(2-propenal, 2-propenoic acid) in 65% w/w ethanol in water was compared with a control formulation of 65% w/w ethanol in water. Firstly, in *in vivo* tests on human hands as a skin antiseptic, results shown in Table 5A; secondly, the test formulation was assessed by the Modified Kelsey Sykes Test, results shown in Table 5B:

## Formulation

- (a) 1.5% w/w polymer in 65% w/w ethanol (Test)  
 (b) 65% w/w ethanol in water (Control)

Table 5A

<u>t(hours)</u>	<u>(a) (counts)</u>	<u>(b) (counts)</u>
0	11000	800
2	500	3100
4	500	1600

t = 0 (before application)

t = 2,4 (2 hrs and 4 hrs, respectively, after application and hands being gloved)

Table 5B

<u>Organism</u>	<u>Initial Count (cfu/ml)</u>	<u>Fraction Negative Tubes</u>
<i>S.aureus</i>	$7.0 \times 10^8$	5/5
<i>E.coli</i>	$1.6 \times 10^9$	5/5
<i>Ps.aeruginosa</i>	$4.6 \times 10^8$	5/5
<i>P.vulgaris</i>	$1.6 \times 10^8$	5/5

**Example 6**

A series of tests were conducted to examine the impact of poly(2-propenal, 2-propenoic acid) on the odours of compositions containing phenols and/or glutaraldehyde. Results are shown in Table 6:

5    **Solution A**    -    1g poly(2-propenal, 2-propenoic acid) was dissolved in 50mL of 1% w/w sodium carbonate.

**Solution B**    -    1g of 4-tert-amylphenol and 2g of sodium hydroxide was dissolved in 40mL of water.

**Solution C**    -    25% w/w glutaraldehyde in water.

10

**Table 6**

<u>Sample</u>	<u>1 minute</u>	<u>5 minutes</u>
Solution A + Water	15 min	little or no odour
Solution B + Water	15 min	medium phenolic odour
Solution C + Water	15 min	strong pungent, irritating odour
Solution B + Solution A	15 min	little or no phenolic odour
Solution C + Solution A	15 min	medium pungent, irritating odour

**Example 7**

A series of tests were conducted to examine any synergy between the antimicrobial activity of solutions containing poly(2-propenal, 2-propenoic acid)

15    and EDTA and/or phenols, and/or isothiazolinones, and/or glutaraldehyde.

If the Minimum Kill Concentrations of compounds A, B, and a mixture of A and B are a, b and m, respectively, then there is synergy ("S") upon mixing A and B if

$$a/m + b/m > 1$$

ie.  $S = (a + b)/m > 1$  for synergy

- 5 The following solutions were tested by the Minimum Kill Concentration Test, and gave the results shown in Table 7:

Table 7

concentrations in p.p.m.

	<u>Experiment 1</u>	<i>S.aureu</i> s	<i>Ps.aeruginosa</i>	<i>E.coli</i>	<i>P.vulgaris</i>	<i>B.cereus</i>	<i>C.albicans</i>	<i>A.niger</i>
a	polymer	250	250	125	65	65	125	500
b	EDTA	625	625	80	155	80	155	1250
m	polymer+EDTA	250	500	250	125	30	125	1000
S		4	2	1	2	5	2	2
	<u>Experiment 2</u>	<i>S.aureu</i> s	<i>Ps.aeruginosa</i>	<i>E.coli</i>	<i>P.vulgaris</i>	<i>B.cereus</i>	<i>C.albicans</i>	<i>A.niger</i>
a	polymer	40	625					
b	glutaraldehyde	80	155					
m	polymer+ glutaraldehyde	<20	310					
S		>6	3					
a	polymer	<10	625					
b	phenol	20	>10000					
m	polymer+ phenol	<20	1250					
S		>1	>9					

a	polymer		625				625
b	thiazolinone		1000				60
m	polymer+ thiazolinone		625				80
S			3				9

phenol = DOWICIDE A; thiazolinone = KATHON

### Example 8

The effects of the presence of poly(2-propenal, 2-propenoic acid) on the migration of various agents across a model for skin were studied as follows.

5 Results are shown in Table 8:

- 10 (a) poly(2-propenal, 2-propenoic acid) (0.5 g) was dissolved in polyethylene glycol 1000 (10 g) by stirring at 70°C, then sodium hydroxide micro-pellets (50 mg) were added and stirred for 2 minutes, and then octyl methoxy cinnamate (10 g; sunscreen agent) was added; followed by a mixture of the polymeric emulsifiers PEMULIN TR1 and CARBOPOL 2984 (0.5g; equal parts) whilst maintaining the temperature at 70°C/15 minutes. This resulting composition was then poured with stirring into water (79 g; at room temperature) and then the pH adjusted to 7.
- 15 (b) The same as (a) above, except the octyl methoxy cinnamate was substituted by octyl dimethyl p-aminobenzoate.
- (c) The same as (a) above, except the 1 : 1 mixture of polymeric emulsifiers were substituted by a 1 : 1 mixture of TWEEN 80 and stearic acid.
- 20 (d) The same as (a) above, except the 1 : 1 mixture of polymeric emulsifiers were substituted by a 1 : 1 mixture of TWEEN 80 and stearic acid - followed by CARBOPOL 2984 (0.25 g).

- (e) The same as (a) above, except the poly(2-propenal, 2-propenoic acid) was omitted.
- (f) The same as (c) above, except the poly(2-propenal, 2-propenoic acid) was omitted.

5 In a special apparatus, the samples were applied to one side of a 0.45 micron cellulose acetate membrane in contact with, on the other side, a stirred solution of ethanol. "After" 1.5 hours, the spectrum of the "ethanol" was compared with the "Before" solution of sample in the same solvent:

Table 8

10 "yes" = exhibits max; "no" = does not

<u>Sample</u>	<u>Before</u>	<u>After</u>
octyl methoxy cinnamate	max = 310 nm	
octyl dimethyl p-aminobenzoate	max = 315 nm	
8(a)	yes	no
8(b)	yes	no
8(c)	yes	no
8(d)	yes	no
8(e)	yes	yes
8(f)	yes	yes

Example 9

The capacity of poly(2-propenal, 2-propenoic acid) to absorb free radicals is indicated by each of the following:

Firstly a solution of 1% w/w poly(2-propenal, 2-propenoic acid) in aqueous sodium carbonate, adjusted to pH 7.1, was treated with two successive additions of Fenton's reagent comprising 0.1% w/w ferrous sulphate (3 mL) and 30% v/v hydrogen peroxide (3 mL), and stirred; bubbles of oxygen were observed and the  
 5 typical golden colour of the solution of poly(2-propenal, 2-propenoic acid), disappeared.

Secondly, the following were mixed, as shown in Table 9:

Table 9

	Sample 1	Sample 2
Linseed oil (12.5 g)	yes	yes
Light petroleum bp 70-90°C (25 g)	yes	yes
Ethanol (20 g)	no	yes
Polymer (0.25 g) in ethanol (20 g)	yes	no
Cobalt (II) acetate (1%) in ethanol (0.1 g)	yes	yes

10

Yes = included in sample

No = NOT included in sample

15

The inhibition by the poly(2-propenal, 2-propenoic acid) of the cobalt catalysed free-radical autoxidation of the linseed oil was demonstrated by the speed of each of the samples becoming "tacky" after drawing as films upon glass, namely,  
 sample 2 > sample 1.

#### Example 10

Shown in Tables 10A, 10B and 10C are results obtained for the following typical compositions derived from the present invention:

(a) poly(2-propenal, 2-propenoic acid) (18 g) was dissolved in water (528 g) containing sodium hydrogen carbonate (18 g), and then a mixture of tetrasodium EDTA (18 g) and sodium lauryl sulphate (18 g) was added, and stirring continued for 30 minutes, after which the pH was adjusted from 8.5 to 9 by the addition of sodium hydroxide micro-pellets (approximately 2 g).

(b) Part A: poly(2-propenal, 2-propenoic acid) (2.7 g) was dissolved by stirring in water (63 g) containing sodium carbonate (0.9 g), DOWFAX 3B2 (2.7 g) was added and stirring continued for 15 minutes; the pH was adjusted from 9.4 to 5.1 by the addition of 10% w/w hydrochloric acid (2.5 g).

Part B: Water (27 g) containing sodium carbonate (1.35 g) and tetrasodium EDTA (2.7 g).

Part B was added to Part A, immediately before the microbiological test.

(c) poly(2-propenal, 2-propenoic acid) (2 g) was dissolved by stirring for 20 minutes in water (98 g) containing DOWICIDE A (2 g); DOWFAX 3B2 (8 g) was added and stirring was continued for 60 minutes to give a clear solution of pH 10.5 which was then adjusted by the addition of 10% w/w hydrochloric acid (1.2 g) to a stable emulsion of pH 5.0. The antimicrobial results were obtained after aging 38°C/14 days.



Table 10A

## Modified Kelsey Sykes Test:

<u>Sample</u>	<u>Organism</u>	<u>Inoculum (ctu/mL)</u>	<u>Fraction negative tubes</u>
10(a)	<i>E.coli</i>	$5.3 \times 10^6$	5/5
	<i>S.aureus</i>	$4.2 \times 10^8$	4/5
	<i>Ps. aeruginosa</i>	$4.5 \times 10^8$	5/5
	<i>P.vulgaris</i>	$2.7 \times 10^8$	5/5
10(b)	<i>E.coli</i>	$5.3 \times 10^8$	5/5
	<i>S.aureus</i>	$3.8 \times 10^9$	5/5
	<i>Ps.aeruginosa</i>	$4.2 \times 10^8$	5/5
	<i>P.vulgaris</i>	$3.3 \times 10^8$	5/5
10(c)	<i>E.coli</i>	$4.3 \times 10^9$	5/5
	<i>S.aureus</i>	$4.9 \times 10^9$	5/5
	<i>Ps.aeruginosa</i>	$5.8 \times 10^9$	5/5
	<i>P.vulgaris</i>	$2.8 \times 10^8$	5/5

Table 10B5 Sporicidal Test

<u>Sample</u>	<u>Time (hours)</u>	<u>Fraction Negative Tube</u>
10(a)	3	5/5
10(a)	7	5/5
10(a)	24	5/5

Table 10CSporicidal Efficacy Test

cfu/slide

Sample	Control count after 72 hours incubation	Counts of samples after 72 hours incubation
11(b)	$3.4 \times 10^4$	<10
11(c)	$3.4 \times 10^4$	<10

- 5 Modifications and variations such as would be apparent to the skilled addressee are considered to fall within the scope of the present invention.

CLAIMS

1. A method for the preparation of compositions of poly(2-propenal, 2-propenoic acid), whereby the compositions exhibit one or more of the properties of increased stability, increased antimicrobial activity, reduced  
5 trans-dermal migration of low molecular weight components of the composition, and the formation of continuous antimicrobial film on substrates, the property or properties making the composition suitable for one or more of antimicrobial use, dermatological use, and/or use as an animal feed additive.
- 10 2. A method according to Claim 1 wherein the composition exhibits increased stability, the method comprising the acidification of compositions of poly(2-propenal, 2-propenoic acid) to which has been added one or more anionic surfactant and/or one or more phenol.
- 15 3. A method according to Claim 2 wherein the composition containing poly(2-propenal, 2-propenoic acid) is firstly stored in basic composition.
4. A method according to Claim 2 or 3 wherein the anionic surfactant is selected from either sodium lauryl sulphate or disodium decyl (sulfophoxy) benzene sulfonate and disodium oxybis (decylsulfophenoxy) benzene sulfonate.
- 20 5. A method according to Claims 2 to 4 wherein the phenol is o-phenyl-phenol.
6. A method according to Claim 1 wherein the composition exhibits increased antimicrobial activity, the method comprising preparation of the poly(2-propenal, 2-propenoic acid) in the presence of air and/or oxygen, with or without inhibitor.
- 25 7. A method according to Claim 1 wherein the composition exhibits increased antimicrobial activity, the method comprising the making of the composition of poly(2-propenal, 2-propenoic acid) into a basic composition.

8. A method according to Claim 7 wherein the composition further comprises one or more of the group: ethylene diamine tetra acetic acid, a lower alkanol, a phenol, isothiazolinones and glutaraldehyde.
9. A method according to Claim 1 wherein the composition further comprises  
5 phenols and/or glutaraldehyde, whereby the odour of the phenols and/or glutaraldehyde is reduced by the presence of the poly(2-propenal, 2-propenoic acid).
10. A method according to Claim 1 wherein the composition exhibits reduced  
10 trans-dermal migration of low molecular weight components of the composition as a result of the presence of poly(2-propenal, 2-propenoic acid).
11. A method according to Claim 10 wherein the low molecular weight composition contains a sunscreen agent.
12. A method according to Claim 11 wherein the sunscreen agent is either or  
15 both of octyl methoxy cinnamate and octyl dimethyl p-aminobenzoate
13. A method according to Claim 1 wherein the composition, especially for dermatological use, exhibits a suncreening effect as a result of the presence of poly(2-propenal, 2-propenoic acid).
14. A method according to Claim 1 wherein the composition exhibits the  
20 formation of a continuous antimicrobial film on substrates, the method comprising the combination of poly(2-propenal, 2-propenoic acid) together with anionic surfactant and/or a phenol.
15. A method according to Claim 14 wherein the surfactant is sodium lauryl sulphate or disodium decyl (sulfophoxy) benzene sulfonate and disodium  
25 oxybis (decylsulfophenoxy) benzene sulfonate.
16. A method according to Claim 14 wherein the phenol is o-phenyl-phenol.

17. A method according to Claim 1 wherein the composition, especially for dermatological use exhibits a free-radical screening effect as the result of the presence of poly(2-propenal, 2-propenoic acid).
- 5 18. A composition adapted for antimicrobial uses and/or dermatological uses and/or uses as an animal feed additive, the composition comprising poly(2-propenal, 2-propenoic acid) and one or more of the following: an anionic surfactant, a phenol, ethylene diamine tetra acetic acid, a lower alkanol, isothiazolinones, glutaraldehyde, sunscreen agent, and an absence of low molecular weight components of the composition.
- 10 19. A composition according to Claim 18, wherein the composition comprises an anionic surfactant chosen from either sodium lauryl sulphate or disodium decyl (sulfophoxy) benzene sulfonate and disodium oxybis (decylsulfophenoxy) benzene sulfonate.
20. A composition according to Claim 18 wherein the phenol is o-phenyl-phenol.
- 15 21. A composition according to Claims 18 to 20 wherein the composition is an emulsion.
22. The use of compositions according to any one of Claims 18 to 21 for antimicrobial uses.
23. The use of compositions according to any one of Claims 18 to 21 for  
20 dermatological uses.
24. The use of compositions according to any one of Claims 18 to 21 as an animal feed additive.
- 25 25. A method for the preparation of compositions of poly(2-propenal, 2-propenoic acid) substantially as hereinbefore described with reference to any one or more of examples 1 to 10.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00578

## A. CLASSIFICATION OF SUBJECT MATTER

Int Cl<sup>6</sup>: A61K 31/765, 7/42, 7/48; C08F 16/34, 16/38; C08L 29/00, 29/14; A01N 35/02; A61L 2/18

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC A61K and keywords below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DERWENT, STN INTERNATIONAL, FILE CAPLUS. KEYWORDS: ACROLEIN, POLY (2-PROPENAL, 2-PROPENOIC ACID), POLYMER, SUNSCREEN, DERMATOLOGICAL, ANTIMICROBIAL, ANTIBACTERIAL, BIOSTATIC, BIOCIDAL, ANIMAL FEED.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/38186 A (CHEMEQ PTY LIMITED) 5 December 1996 (see whole document)	18
X	JP 6-72954 (NIPPON SHOKUBAI CO LTD) 15 March 1994 (see whole document)	1
X	AU 11686/95 A (DEGUSSA AKTIENGESELLSCHAFT) 24 August 1995 (see whole document)	1

☒ Further documents are listed in the continuation of Box C

☒ See patent family annex

\* Special categories of cited documents:

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- \*Z\* document member of the same patent family

Date of the actual completion of the international search

18 August 1999

Date of mailing of the international search report

20 SEP 1999

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 99/00578

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88/04671 A (BIOPOLYMERS LIMITED) 30 June 1988 (see whole document)	1

# INTERNATIONAL SEARCH REPORT

## Information on patent family members

International application No.  
PCT/AU 99/00578

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	96/38186	AU	57557/96	EP	828498		
JP	6-72954	NONE					
AU	11686/95	CA	2142220	JP	7242710	ZA	9501114
		DE	4404404	NO	950512	EP	667358
		NZ	270467				
WO	88/04671	AU	10864/88	DE	3789150	HK	1033/94
		NO	883639	CA	1320128	DK	4697/88
		IN	166555	CN	87108390	EP	339044
		JP	2-501750				

END OF ANNEX